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BRIEF COMMUNICATION

Regional variation in CCR5-Δ32 gene distribution among women from the US HIV Epidemiology Research Study (HERS)

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The CCR5-Δ32 genotype is known to influence HIV-1 transmission and disease. We genotyped 1301 US women of various races/ethnicities participating in the HIV Epidemiologic Research Study. None was homozygous for CCR5-Δ32. The distribution of heterozygotes was similar in HIV-1 infected and uninfected women. Thirty-seven (11.8%) white, 28 (3.7%) blacks/African Americans (AA), seven (3.3%) Hispanics/Latinas, and one (6.6%) other race/ethnicity were heterozygous. The frequency of heterozygotes differed among sites for all races combined (P = 0.001). More heterozygotes were found in AA women in Rhode Island (8.9%) than in the other sites (3.1%) (P = 0.02), while heterozygosity in white women was most common in Maryland (28.6%) (P = 0.025). These regional differences could be accounted for by racial admixture in AAs, but not in whites. Regional variations should be considered when studying host genetic factors and HIV-1 in US populations.
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Polymorphism in the β chemokine receptor gene CCR5 affects HIV-1 entry, transmission, and outcome.[1–3] Most people homozygous for a CCR5 gene variant (the 32 bp deletion (Δ32/Δ32)) are highly resistant to HIV-1 infection.[3] HIV-infected individuals heterozygous for CCR5 (+/Δ32) have about a 2-year delay in progression to AIDS and slower CD4+ T cell decline compared with HIV infected individuals who do not carry the Δ32 polymorphism.[4–6] We have examined the distribution of this gene in a cohort of United States (US) women known as the HIV Epidemiology Research Study (HERS). This is a prospective, multisite study conducted to define the epidemiologic, biologic, psychological, and social effects of HIV-1 infection on the health of US women and to examine the progression of HIV-1 disease.[7] The HERS study focuses on women of various races infected with or at high risk for HIV through heterosexual transmission and/or through illicit drug injections. Seropositives and negatives were matched by both distribution of race and by risk behavior in the HERS study design. A total of 1310 HIV infected and uninfected women aged 16 to 55 years old were recruited between April 1993 and January 1995 at four study sites: New York (NY), Michigan (MI), Maryland (MD), and Rhode Island (RI). Two of these sites (MI, RI) recruited primarily from medical care/drug abuse therapy settings, and two (NY, MD) from community sources. At all sites, HIV-1 uninfected women were recruited over the same time period and from the same or comparable sources as the HIV-1 infected women. The Institutional Review Boards at each institution approved the study.[7] Baseline data on participant characteristics were previously published.[7,8] Here, we report the distribution of the CCR5-Δ32 gene in these women, by race/ethnicity, study site, and HIV-1 status.

Racial admixture was defined as the presence of one or more parents or grandparents reported as being of a different race than the self-identified racial or ethnic group of the study participant. No information on the race or ethnicity of all six parents and grandparents was treated as missing data for admixture (ie, admix-

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ture could be underestimated). The proportions of missing admixture data were compared among the sites by the χ^2 test. Chi-squared or two-tailed Fisher's exact test was used, when appropriate, to assess the distribution of genotypes in the overall cohort, within racial groups and sites, and in HIV-1 infected and uninfected women using SAS version 6.12 (SAS Institute, Cary, NC, USA) and Epi-info (CDC, Atlanta, GA, USA). A *P*-value of less than 0.05 was considered significant. The sample sizes of Hispanic, Asian, and Native American groups were not large enough to provide statistical power for stratification by site.

Genomic DNA was extracted from peripheral blood mononuclear cells (PBMC) by the Puregene method (Gentra Systems Minneapolis, MN, USA). Detection of CCR5-Δ32 by restriction fragment length polymorphism was performed using in house primers: 5′-CCTGGCTGTCGTCCATGCTG-3′ and 5′-CTGATCTAG AGCC AT GTGCACAACTCT-3′. The PCR product was digested with EcoR1, which resulted in two bands of 332 and 403 bp for the homozygous CCR5/CCR5 [+/+], two bands of 332 and 371 bp for the homozygous CCR5-Δ32/Δ32 [Δ32/Δ32], and three bands of 332, 371, and 403 bp for the heterozygous CCR5/Δ32 [+/Δ32].

We excluded from analysis six individuals whose DNA was depleted and three whose DNA failed to amplify. Of the 1301 women genotyped for CCR5-Δ32, 761 (58.5%) identified themselves as black/African American (AA), 314 (24.1%) as white, 211 (16.2%) as Hispanic/Latina, 13 (1%) as Native American, and two (0.2%) as Asian. Regarding HIV status, 422 women (32.4%) were HIV-1 uninfected and 879 (67.6%) were HIV-1 infected. Of the HIV-1 infected women, 865 (98.4%) were HIV seropositive at enrollment and 14 (1.6%) seroconverted during the study. For this genetic analysis, HIV-1 seroprevalent and seroconverting women were considered as one group. None of the individuals in this study were homozygous for CCR5-Δ32, 73 (5.6%) were heterozygous for CCR5 (+/Δ32), and 1228 (94.4%) were homozygous for CCR5 (+/+). The distribution of the genotypes in the overall cohort was in Hardy-Weinberg equilibrium (HWE) (Guo and Thompson HWE exact test;[9] $P = 0.62$). Within each racial group and in the HIV-1 infected and uninfected groups, the genotypes of the women were also distributed according to the HWE.

The racial/ethnic group distribution (in %) of CCR5-Δ32 heterozygosity was as follows: AA, 3.7% (28/761); white, 11.8% (37/314); Hispanic/Latina, 3.3% (7/211); and other 6.6% (1/15). The frequency of CCR5 heterozygotes in white women was similar to that found in published data.[1,3,10] Among the AA women this frequency was similar to that reported in high risk AAs by Dean *et al*[3] (3.4%) but lower than that found in AA blood donors by Zimmerman[10] (5.8%), although the difference was not statistically significant.

The CCR5-Δ32 heterozygous genotype has been reported, although inconsistently, to influence HIV-1 transmission in adults.[1,3,4,10–12]. We examined CCR5-Δ32 frequencies in all racial groups in relation to HIV-1 status. There was no significant difference in the frequency of CCR5 genotypes between HIV-1 infected and HIV-1 uninfected women in the Hispanic/Latina, Asian, Native American (data not shown), AA or white groups (Table 1), which is consistent with findings from other studies in adults[3,4,10,11] showing no association with HIV-1 infection.

We then examined whether heterozygosity differed in frequency by site among AAs and whites (groups with sufficient numbers to compare by site). The distribution of the CCR5 genotypes among AAs at the four sites suggested differences among the four sites (overall χ^2 test for heterogeneity = 6.801, $P = 0.08$). Investigation of the site data revealed the frequency of heterozygotes to be significantly higher among AAs from Rhode Island (8.9%) compared with the AA frequency in the other three sites combined (3.1%; $\chi^2 = 5.15$, $P = 0.02$) (Table 2). The CCR5 heterozygosity rate was significantly higher (two-tailed Fisher's test, $P = 0.03$) than previously reported for high risk AAs but not significantly different from the frequency in AA blood donors.[10] The frequency of heterozygotes in whites from RI (11.9%) was no different than that observed in the other three sites combined (11.5%) ($\chi^2 = 0.01$, $P = 0.9$), nor was the frequency of heterozygotes different in all non-AAs from RI (10%) compared to non-AAs from the other three sites combined (6.8%) ($\chi^2 = 1.82$, $P = 0.18$). Thus, the high frequency of CCR5 (+/Δ32) in AA from RI explains the high frequency of the genotype in all women at the RI study site.

A possible explanation for this higher frequency among AAs in RI could include a higher rate of white racial admixture among AA women in RI compared with those at other sites. For those who had available parental/and or grandparental lineage data, we found that admixture of AAs with other races significantly differed among sites (overall χ^2 for heterogeneity = 45.14, $P < 0.0001$). In RI, this admixture was observed in 37 of 58 (63.8%) individuals compared to 56 of 186 (30.0%) women in MI, 53 of 233 (22.7%) in MD, and 20 of 109 (18.3%) in NY (RI *vs* three other sites $\chi^2 = 39.9$; $P < 0.0001$). The percentages of missing data on admixture in AA women were not significantly different among sites. The admixture rate in RI appears to be high, although it may be limited by incorrect reporting of parental and grandparental race.

We also found the distribution of CCR5 heterozygotes among whites suggested differences exist among the sites (χ^2 for heterogeneity among sites in whites, 8.50; $P = 0.037$). Maryland had the highest frequency of heterozygotes (28.6%, 6/21), which was significantly higher than that observed in RI, MI and NY whites combined (10.6%, 31/293) (two-tailed Fisher's test, $P = 0.025$).

We determined the presence of racial admixture in whites. Four of 15 (26.7%) individuals in MD were admixed with other races, compared to 4/33 (12.1%) in NY, 12/152 (7.9%) in RI, and 1/30 (3.3%) in MI. The 4×2 χ^2 for heterogeneity of racial admixture in whites among sites was not possible because of small expected values, but racial admixture in MD (26.7%) was higher than in the three other sites combined (7.9%) (two-tailed Fisher's test, $P = 0.037$). However, racial admixture of whites in MD was not associated with heterozygosity in the direction that would explain higher rates of heterozygosity in this group. Admixture of white with other races should result in a lower frequency of heterozygous persons as we know that the presence of the CCR5-Δ32 polymorphism is lower in AAs and

Table 1 CCR5 genotype frequencies by HIV status within race/ethnic group

Race/ethnicity	*HIV status*	*Total*	*+/Δ32 n (%)*	*+/+ n (%)*	$\chi^{2\,a}$	P
Black/African American (AA)	Positive	536	20 (3.7)	516 (96.3)		
	Negative	225	8 (3.6)	217 (96.4)		
	Total	761	28 (3.7)	733 (96.3)	0.01	NS
White	Positive	182	24 (13.2)	158 (86.8)		
	Negative	132	13 (9.8)	119 (90.2)		
	Total	314	37 (11.8)	277 (88.2)	0.82	NS
All races/ethnicities[b]	Positive	879	50 (5.7)	829 (94.3)		
	Negative	422	23 (5.5)	399 (94.5)		
	Total	1301	73 (5.6)	1228 (94.4)	0.03	NS

[a]Comparison of CCR5 heterozygous frequency in HIV positive *vs* HIV negative women. NS = not significant, $P > 0.05$. [b]All races/ethnicities include Hispanic/Latina, Asian, Native American in addition to AA and white.

Table 2 Distribution of CCR5 genotypes among sites when stratified by race/ethnicity

Race/ethnicity	*Genotypes*							
	+/Δ32	+/+	+/Δ32	+/+	+/Δ32	+/+	+/Δ32	+/+
	RI n (%)		*MD n (%)*		*MI n (%)*		*NY n (%)*	
All races combined[a] (*n* = 1301)	33 (9.7)	306 (90.3)	17 (5.2)	307 (94.8)	9 (3.0)	291 (97.0)	14 (4.1)	324 (95.9)
AA[b] (*n* = 761)	7 (8.9)	72 (91.1)	10 (3.4)	287 (96.6)	7 (2.9)	239 (97.1)	4 (2.9)	135 (97.1)
White[c] (*n* = 314)	24 (11.9)	177 (88.1)	6 (28.6)	15 (71.4)	2 (4.1)	47 (95.9)	5 (11.6)	38 (88.4)

[a]All races combined, RI *vs* three other sites combined; Pearson $\chi^2 = 14.72$, $P = 0.002$. [b]In AA, RI *vs* three other sites combined; Yates' continuity-corrected $\chi^2 = 5.15$, $P = 0.02$. [c]In white, MD *vs* three other sites combined; two-tailed Fisher's test, $P = 0.025$.

other ethnic groups. There was no significant difference in the percentages of missing data on admixture among sites in whites.

We did not perform a quantitative genetic assessment of admixture in this study. Instead we did a qualitative measurement by determining the frequency of study subjects whose parents' or grandparents' racial/ethnic category differed from the self-classified race/ethnicity of the participants. Estimates of racial admixture can be obtained by other methods. For example, using Bernstein's formula[13] and a representative CCR5-Δ32 allele frequency of 0.092 in Caucasians[1] and an intermediate frequency of 0.007[14] in Africans[1,14–17] we derived an estimate of white admixture in the RI AA population of 43.5%. This was less than what we determined by self-assessment (63.8%), but both methods suggest a significant degree of admixture in this population. Williams *et al*[13] have shown in their example that Bernstein's formula and self-reported admixture approaches, given their limitations, are valid qualitative measurement of racial admixture. While determining if racial admixture influenced CCR5 gene distribution in US populations was not the primary goal of the HERS study, these preliminary findings suggest that racial admixture may be a possible explanation for the observed differences among the sites.

Another factor that should be considered is the impact of migration to or within the US. The well-known geographic variation of CCR5-Δ32 frequencies in Europe and Eurasia, with highest frequencies in Northern Europe,[18,19] together with preferential migration of certain European populations to certain US cities or states could influence regional frequencies of CCR5, particularly among US whites. Unfortunately, the questionnaire administered to the participants of this study did not collect information on the geographic origin of their ancestors. The finding that CCR5-Δ32 frequencies vary regionally in the US is not unexpected, as regional variation in the frequency of genes such as HLA has been demonstrated in AAs[20–22] and whites[23] in the US.

Overall, our findings suggest that regional differences in CCR5-Δ32 distribution exist in AA and in white populations in the US. As the HIV epidemic in the US is increasingly concentrated in non-white populations/racial groups,[24] studies of the impact of host genetics on HIV-1 acquisition and disease progression will need to consider the finding that CCR5 gene frequencies differ not only between but also within racial groups.

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